ANTIFUNGAL COMPOSITION

The present invention relates to the treatment of human mycotic infections. It concerns antifungal compositions for use in the treatment of mycotic infections caused by azole resistant yeast strains, comprising terbinafine with an azole 14α-methyldemethylase inhibitor such as the azole fluconazole and/or itraconazole.

Although they are often present as benign commensal organisms in the digestive tract of healthy individuals, fungi, particularly Candida species produce a broad range of serious illnesses in compromised hosts. Such infections are clearly on the rise. Oropharyngeal candidiasis is the most common fungal infection in patients with human immunodeficiency virus (HIV) infection. With the introduction of azole antifungal agents that are bioavailable after oral administration, the approach to the treatment of serious Candida infections is possible. Ketoconazole, the first of these agents to become available, was quickly found to be efficacious in the setting of chronic mucocutaneous candidiasis. However, not long after the introduction of this agent, clinical failure in association with elevated minimum inhibitory concentrations (MICs) of ketoconazole that developed during prolonged therapy were reported. This problem achieved prominence with the subsequent introduction of fluconazole. Fluconazole, a water-soluble triazole with greater than 90 % bioavailability after oral administration, is used extensively to treat a wide range of Candida infections. In particular, it is widely used as therapy for oropharyngeal candidiasis in patients with advanced HIV infection and AIDS. Although oropharyngeal candidiasis usually responds readily to fluconazole, it is difficult to completely eradicate the infection and relapse often occurs within several months following the completion of therapy. For this reason, many AIDS patients receive fluconazole either

continuously on intermittently over long periods of time.

To a greater extent than with other azoles, resistance to fluconazole has developed and is becoming a significant clinical problem, as attested by isolation from, in particular, AIDS patients of numerous Candida strains showing resistance (see e.g. D. Law et al., J.Antimicrob.Chemother. 34 [1994] 659-668).

It has now been found that, surprisingly, a combination of the squalene epoxidase inhibitor terbinafine (Lamisil®) and an azole 14α-methyldemethylase inhibitor such as fluconazole and/or itraconazole is active against azole-resistant fungal strains. By using this combination of compounds there is provided a method for treating human mycotic infections caused by azole-resistant fungal strains.

Suitable azole 14α-methyldemethylase inhibitors are in particular imidazole and triazole antifungal agents.

Preferred imidazole antifungal agents include clotrimazole (Arzneim.-Forsch. 22 [1972] 1280), miconazole (Arzneim.-Forsch. 21 [1971] 256; econazole (Arzneim.-Forsch. 25 [1975] 224); isoconazole (Arzneim.-Forsch. 29 [1979] 1344); trioconazole (Antimicrobial Agents Chemotherapy 15 [1979] 597-602); sulconazole (Eumycetes and Mycosis 23 [1982] 314-317; oxiconazole (Arzneim.-Forsch. 32 [1982] 17-24); cloconazole (J.Med.Chem. 26 [1983] 768-770]); bifonazole (Arzneim.-Forsch. 33 [1983] 517-524); butoconazole (J.Med.Chem. 21 [1978] 840; fenticonazole (Arzneim.-Forsch. 31 [1981] 2127); zinoconazole (J.Med.Chem. 26 [1983] 442-445) and ketoconazole (J.Med.Chem. 22 [1979] 1003-1005).

Preferred triazole antifungal agents include terconazole (J.Med.Chem. 26 [1983] 611-613); itraconazole (Antimicrobial Agents and Chemotherapy 26 [1984] 5-9); vibunazole (Arzneim.-Forsch. 33 [1983] 546); fluconazole

(Antimicrobial Agents and Chemotherapy 27 [1985] 815-818), and (R)(-)-α-(4-chlorophenyl)-α-(1-cyclopropyl-1-methyl-ethyl)-1H-1,2,4-triazol-1-ethanol in free form or in salt or metal complex form (GB 2'161'483) (hereinafter briefly referred to, in free form, as "compound A").

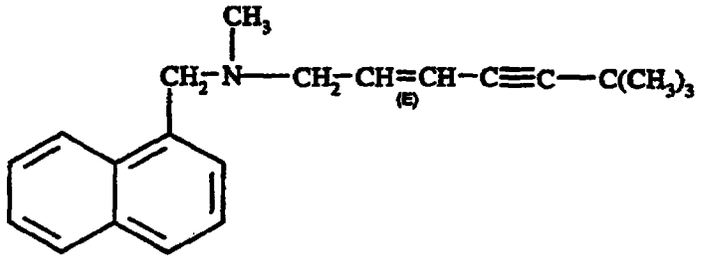
Especially preferred azoles are itraconazole and fluconazole.

Not all combinations of antifungal drugs show synergistic or even additive effects, and even antagonistic effects have been reported in the literature. Thus in e.g. E. Martin et al., Antimicr. Agents and Chemother. 38 [1994] 1331-1338, it has been reported that fluconazole antagonizes the candidacidal action of amphotericin B; in Abstr. Ann. Meeting Am. Soc. Microbiol. 87 (1987) 392 it was reported that the use of terbinafine and ketoconazole in Candida albicans produced no enhancement of antimycotic activity; in Eur. J. Clin. Microbiol. Infect. Dis. 7 (1988) 732-735 it was stated that in vitro terbinafine appears to act antagonistically with the azoles; and in Drugs Today 24 (1988) 705-715 it is again mentioned that in combination therapy, not all combinations of antifungal drugs show synergistic or even additive effects.

It appears therefore that combination therapy with antimycotics is highly unpredictable.

Thus, while one might on theoretical grounds expect that combinations of pharmacologically active agents that inhibit a single biosynthetic pathway at two separate steps are normally more active than those which act only on one step, and that combinations of e.g. terbinafine with azoles such as fluconazole and/or itraconazole should possess at least additive activity, it is very surprising that, for unknown reasons, such combinations are still effective even where resistance to the azoles has already developed, namely, synergistic effects are maintained even in situations where the mycotic strains have become resistant to the azoles.

The composition of the invention for treating human mycotic infections caused by azole-resistant fungal strains comprises an azole 14α-methyldemethylase inhibitor such as fluconazole and/or itraconazole, and the arylmethylamine squalene epoxidase inhibitor terbinafine of formula



I

in free base or acid addition salt, e.g. hydrochloride acid addition salt form.

The most preferred azole is fluconazole. Terbinafine preferably is in pharmaceutically acceptable, preferably in hydrochloride salt form. The preferred fungus preferably is a yeast, if preferably is of the genus Candida, it especially is Candida albicans. The azole-resistance induced mycosis may be superficial or systemic, it especially is oropharyngeal. It is deleterious to e.g. the skin or mucosa.

The azole-resistance may be a cross-resistance and involve multiple azoles.

The antifungal composition of the present invention is prepared by incorporating the arylmethylamine of formula I to an azole 14α-methyldemethylase inhibiting antifungal agent, such as fluconazole and/or itraconazole.

The invention thus concerns an antifungal composition for use in the treatment of mycotic infections caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis, comprising terbinafine as defined above and an azole 14α-methyldemethylase inhibitor, such as fluconazole and/or itraconazole.

It further concerns the use of an antifungal composition comprising terbinafine as defined above and an azole 14α-methyldemethylase inhibitor, such as fluconazole and/or itraconazole, in the preparation of a medicament for use in the treatment of mycotic infections caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis in a human subject compromised by such azole-resistance.

It further concerns a process for the preparation of an antifungal composition as defined above, comprising the incorporation of terbinafine as defined above to an azole 14α-methyldemethylase inhibiting antifungal agent, such as fluconazole and/or itraconazole.

It further concerns a method of treatment of an azole-resistant fungal infection caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis, comprising administering a therapeutically effective amount of an antifungal composition as defined above to a patient in need of such treatment.

In the antifungal composition of the present invention the weight ratio of the azole antifungal agent to the arylmethylamine antifungal agent may be varied within a wide range, but is preferably within a range of from 100:1 to 1:500, more preferably from 25:1 to 1:125. By mixing the azole antifungal agent and the arylmethylamine antifungal agent terbinafine in a weight ratio within the above range, an excellent effect is obtainable in the treatment of mycosis caused by azole resistant fungal strains, particularly yeast strains, e.g. Candida strains such as Candida albicans, Candida (= Torulopsis) glabrata, Candida krusei and Candida tropicalis; Cryptococcus strains such as Cryptococcus neoformans; or Trichophyton strains,

e.g. Trichophyton mentagrophytes; especially Candida strains, particularly Candida albicans.

The composition of the present invention can be adapted for local administration and can be incorporated in a usual pharmaceutical carrier in a wide range of concentrations (usually in an amount of from about 0.1 % to about 10 % by weight of the total composition) to prepare a formulation. The composition of the present invention can be used for oral administration in the form of tablets, capsules or a liquid, and it may also be used for non-oral administration such as subcutaneous, intramuscular or intravenous injection. It thus normally is a fixed combination. However, administration of the active agents may also be effected in the form of a free combination, i.e. separately, e.g. sequentially, in any order.

The beneficial activity can be shown in vitro using various azole-resistant strains. The assay is performed with RPMI 1640 medium in 96-well, flat-bottom microdilution plates using a chequered drug dilution system. Terbinafine (in hydrochloride salt form) and the azole are used in concentrations from 100 µg/ml to 0.006 µg/ml. Minimal inhibitory concentrations (MIC) are determined after a 48-hour incubation at 37°C. Minimal fungicidal (MFC) concentrations are evaluated 24 hours after transfer of drug-treated cells to drug-free medium. The endpoints used for MIC determination are either 100 % inhibition (Tables 1 and 4) or (as is usual) 80 % inhibition (Tables 2 and 3) (Terb = terbinafine; Flu = fluconazole; Itra = itraconazole; C. = Candida).

Table 1

In vitro testing of the combination terbinafine/fluconazole against fluconazole-resistant strains of Candida albicans, C. glabrata, C. krusei and C. tropicalis.

Strain	MIC for 100 % inhibition (µg/ml)			
	Alone		Combination	
	Terb	Flu	Terb	Flu
C.albicans	>100	>100	12.5	6.25
C.albicans	>100	>100	12.5	12.5
C.albicans	>100	>100	12.5	6.25
C.albicans	>100	>100	3.13	12.5
C.tropicalis	>100	>100	>100	>100
C.glabrata	>100	>100	3.13	25.0
C.albicans	>100	>100	3.13	50.0
C.krusei	>100	50.0	3.13	50.0

In contrast to exposure of fungi to fluconazole or terbinafine alone, fungal growth was 100 % inhibited in all but one strain when combinations of fluconazole and terbinafine were used. In four of the strains, combinations of both drugs at concentrations <100 µg/ml were fungicidal in action, a result which could not be achieved with either of the drugs alone.

Table 2

In vitro testing of the combination terbinafine/fluconazole against fluconazole-resistant Candida isolates

Strain	MIC for 80% inhibition (µg/ml)			
	Alone		Combination	
	Flu	Terb	Flu	Terb
C.albicans	>16	>1	0.25	0.1
C.albicans	>16	>1	16	0.5
C.tropicalis	>16	>1	>0.125	0.06
C.glabrata	>16	>1	16	0.125

Table 3

In vitro testing of the combination terbinafine/itraconazole against itraconazole-resistant Candida isolates

Strain	MIC for 80% inhibition (µg/ml)			
	Alone		Combination	
	Itra	Terb	Itra	Terb
C.albicans	>4	>1	≤0.03	0.03
C.tropicalis	>4	>1	≤0.03	0.03

Table 4

In vitro testing of the combination terbinafine/compound A against fluconazole-resistant Candida isolates

Strain	MIC for 100 % inhibition (µg/ml)			
	Alone		Combination	
	Compound A	Terb	Compound A	Terb
C.albicans	>100	>100	0.4	3.13
C.albicans	100	>100	0.4	3.13
C.albicans	100	>100	1.56	3.13
C.albicans	100	>100	0.8	3.13

Table 5

Summary of azole-resistant fungal strains in which a combination of terbinafine and an azole was found active and the azole to which resistance occurred

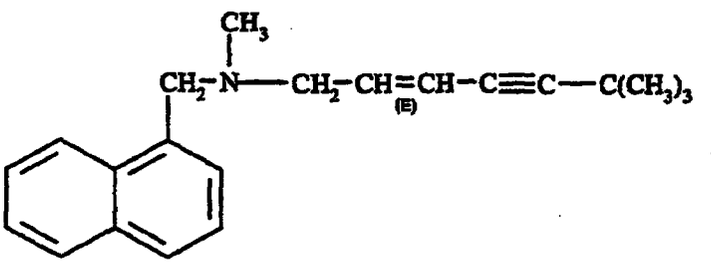
Fungal species	Azole	Proportion of strains showing activity
C. albicans	Fluconazole	5 of 10
C. albicans	Itraconazole	9 of 10
C. tropicalis	Fluconazole	2 of 2
C. krusei	Fluconazole	1 of 4
C. paratropicalis	Fluconazole	1 of 1
C. glabrata	Fluconazole	1 of 2
Cryptococcus neoformans	Fluconazole	3 of 10
C.albicans	Compound A	4 of 4

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Claims:

1. An antifungal composition for use in the treatment of mycotic infections caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis, comprising terbinafine of formula I



in free base or acid addition salt form, and an azole 14α-methyldemethylase inhibitor.

2. Use of an antifungal composition comprising terbinafine as defined in claim 1 and an azole 14α-methyldemethylase inhibitor in the preparation of a medicament for use in the treatment of mycotic infections caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis in a human subject compromised by such azole-resistance.

3. A process for the preparation of an antifungal composition as defined in claim 1, comprising the incorporation of terbinafine as defined in claim 1 to an azole 14α-methyldemethylase inhibitor antifungal agent.

4. A method of treatment of an azole-resistant fungal infection caused by azole-resistant fungal strains for the avoidance or restriction of deleterious azole-resistance induced mycosis, comprising administering a therapeutically effective amount of an antifungal composition as defined in claim 1 to a patient in need of such treatment.

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5. A composition, use, process or method according to one of claims 1 to 4 wherein terbinafine is in the form of the hydrochloride salt.

6. A composition, use, process or method according to one of claims 1 to 4 wherein the azole is fluconazole.

7. A composition, use, process or method according to one of claims 1 to 4 wherein the azole is itraconazole.

8. A composition, use, process or method according to one of claims 1 to 4 wherein the azole is (R)-(-)-α-(4-chlorophenyl)-α-(1-cyclopropyl-1-methylethyl)-1H-1,2,4-triazol-1-ethanol in free form or in salt or metal complex form.

9. A composition, use, process or method according to one of claims 1 to 4 wherein the azole-resistant fungal strain is a yeast strain.

10. A composition, use, process or method according to one of claims 1 to 4 wherein the azole-resistant fungal strain is a Candida strain.

11. A composition, use, process or method according to one of claims 1 to 4 wherein the azole-resistant fungal strain is Candida albicans.

12. A composition, use, process or method according to one of claims 1 to 4 wherein the azole-resistant fungal strain is a Cryptococcus strain.

13. A composition, use, process or method according to any one of claims 1 to 4 wherein the composition is a fixed combination.

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14. A composition, use, process or method according to any one of claims 1 to 4 wherein the composition is a free combination.

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